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Antifertility Effect of Methoxychlor in Female Rats Dose- and Time-Dependent Blockade of Pregnancy

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16. ABSTRACT

Long-term exposure to methoxychlor (MXC), an estrogenic pesticide, produces infertility in rats, and short-term exposure blocks the decidual cell response (DCR). To address the short-term effects of MXC on fertility, the differential effects of MXC dosage and timing of administration (relative to implantation) on several gestational parameters were investigated. When MXC was administered during early pregnancy (Days 1–8), a dose-dependent decline in implantations and uterine weight was seen with no effect on ovarian weight or corpora lutea; MXC reduced serum progesterone at all doses. Preimplantation administration of MXC (Days 1–3 of pregnancy) produced a decline in implantations and uterine weight, while postimplantation dosing (Days 4–8 of pregnancy) increased resorptions to 100%, decreased uterine weight, and reduced serum progesterone without affecting the number of implantations, ovarian weight, or number of corpora lutea. The DCR of pseudopregnancy was inhibited by 500 mg/kg/day MXC when administered either pre- or postimplantation, but 200 mg/kg/day was without effect in either regimen. When hormonally primed, long-term ovariectomized rats were exposed to doses of 500 mg/kg/day, MXC blocked the induced DCR seen in controls. The data show that short-term MXC dosing during early pregnancy produces a dose-related infertility. The blockade of pregnancy by the preimplantation administration of MXC may be mediated by a direct effect on preimplantation uterine development. The fetal resorption seen following postimplantation dosing is considered a manifestation of both reduced serum progesterone and the direct disruption of normal decidual development by MXC.

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Antifertility Effect of Methoxychlor in Female Rats: Dose- and Time-Dependent Blockade of Pregnancy[1,2]

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Antifertility Effect of Methoxychlor in Female Rats: Dose- and Time-Dependent Blockade of Pregnancy. CUMMINGS, A. M., AND GRAY, L. E., JR. (1989). *Toxicol. Appl. Pharmacol.* **97**, 454–462. Long-term exposure to methoxychlor (MXC), an estrogenic pesticide, produces infertility in rats, and short-term exposure blocks the decidual cell response (DCR). To address the short-term effects of MXC on fertility, the differential effects of MXC dosage and timing of administration (relative to implantation) on several gestational parameters were investigated. When MXC was administered during early pregnancy (Days 1–8), a dose-dependent decline in implantations and uterine weight was seen with no effect on ovarian weight or corpora lutea; MXC reduced serum progesterone at all doses. Preimplantation administration of MXC (Days 1–3 of pregnancy) produced a decline in implantations and uterine weight, while postimplantation dosing (Days 4–8 of pregnancy) increased resorptions to 100%, decreased uterine weight, and reduced serum progesterone without affecting the number of implantations, ovarian weight, or number of corpora lutea. The DCR of pseudopregnancy was inhibited by 500 mg/kg/day MXC when administered either pre- or postimplantation, but 200 mg/kg/day was without effect in either regimen. When hormonally primed, long-term ovariectomized rats were exposed to doses of 500 mg/kg/day, MXC blocked the induced DCR seen in controls. The data show that short-term MXC dosing during early pregnancy produces a dose-related infertility. The blockade of pregnancy by the preimplantation administration of MXC may be mediated by a direct effect on preimplantation uterine development. The fetal resorption seen following postimplantation dosing is considered a manifestation of both reduced serum progesterone and the direct disruption of normal decidual development by MXC. © 1989 Academic Press, Inc.

The pesticide methoxychlor (MXC) has toxic effects on the female reproductive system (Bal and Mungkornkarn, 1978) and exhibits weak estrogenic activity both *in vivo* (Welch *et al.*, 1969) and *in vitro* (Bulger *et al.*, 1985). Experiments in which rats received MXC both before and during pregnancy revealed a blockade of implantation, a lack of corpora lutea, and atresia of ovarian follicles (Bal and Mungkornkarn, 1978). However, effects of MXC on ovulation, fertilization, or ovum transport rate were not ruled out in that study.

In a previously reported study (Cummings and Gray, 1987), data demonstrating a dose-related inhibitory effect of MXC on the decidual cell response (DCR) of the uterus suggested that this suppression of decidualization may be responsible, at least in part, for the antifertility effects of the compound

[1] The research described in this article has been reviewed by the Health Effects Research Laboratory, U.S. Environmental Protection Agency, and approved for publication. Approval does not signify that the contents necessarily reflect the views and policies of the Agency nor does mention of trade names or commercial products constitute endorsement or recommendation for use.

[2] Presented in part at the 27th Annual Meeting of the Society of Toxicology, Dallas, TX, February 1988 (*Toxicologist* **8**, 236).

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which are well documented (Gray *et al.*, 1986; Khera *et al.*, 1978; Harris *et al.*, 1974). These data on DCR inhibition identified a strictly maternal effect of MXC, indicated a blockade of implantation, and suggested that the compound's effect on the uterus was independent of potential effects on ovarian function. Further questions remained, however, concerning whether MXC administration would similarly suppress uterine decidualization in bred rats, thus preventing implantation, and whether the preimplantation development of uterine receptivity was more or less sensitive to the pesticide's effects than the postimplantation period of uterine decidual growth.

Through the use of a protocol in which rats received MXC only following successful mating or during pseudopregnancy with DCR induction, the current study was designed to examine the effect of MXC specifically on early pregnancy and implantation, with attention to a variety of ovarian functional parameters. Additional experiments in which animals received MXC strictly during pre- or postimplantation periods were performed to investigate the mechanism by which the pesticide exerts its antifertility effects and to examine the relationship between specific maternal toxicity and early pregnancy maintenance. The decidual cell response, a technique that mimics the response of the uterus to implanting blastocysts and results in the growth of decidual tissue similar to that of early pregnancy (De Feo, 1967), was used in pseudopregnant rats to define direct effects of MXC on uterine function independent from possible effects on fertilized ova and embryonic development.

METHODS

General. Female Holtzman rats were obtained from Small Animal Supply Company (SASCO) at 60 days of age. Body weights ranged from 204 to 254 g at the start of each experiment. Animals were housed in pairs in clear plastic cages (20 × 25 × 47 cm) with heat-treated laboratory-grade pine shavings (Northeastern Products Corp., Warrensburg, NY) as litter. Purina Rodent Chow (5001) and water were given *ad libitum*. A photoperiod of 14 hr of light and 10 hr of darkness was maintained, with the lights on at 5 AM. The temperature in the animal room was 20–24°C with a relative humidity of 40–50%.

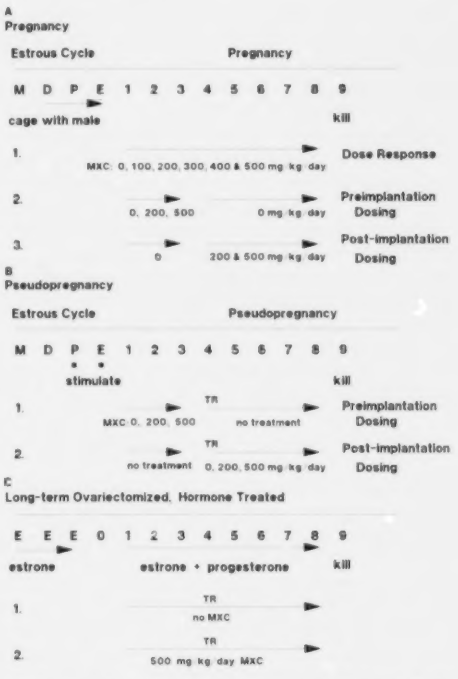

FIG. 1. Animal treatment protocols. Estrous cycles were monitored daily. The initiation of pregnancy and pseudopregnancy was as indicated. Dosing regimens are described under Methods. TR, decidual induction.

Early pregnancy protocol. Estrous cycles of all animals were monitored throughout all experiments by daily vaginal smears, and only those rats exhibiting two consecutive 4- or 5-day cycles were used. Animals were bred by caging virgin females for two consecutive nights prior to estrus with untreated, proven-fertile males (Fig. 1A). Day 0 was defined by a sperm-positive vaginal smear, coinciding with the day of estrus, and the finding of vaginal plugs. In the first experiment, MXC[3] was administered by oral gavage to pregnant rats from Day 1 through Day 8 (Fig. 1A-1). MXC was dissolved in and diluted with

[3] Laboratory-grade methoxychlor (MXC), approximately 98% (Lot No. 124F-5028); estrone (Lot No. 32F-0258); progesterone (Lot No. 123F-0110); and sesame oil (Lot No. 105F-0584) were purchased from Sigma Chemical Company, St. Louis, MO.

sesame oil. Each group of rats received MXC at doses of 0 (vehicle), 100, 200, 300, 400, or 500 mg/kg/day using dilutions to provide equal volumes of solution per kilogram of body weight. This dosing regimen is similar to that previously used in pseudopregnant rats where 100 mg/kg/day was without effect, 500 mg/kg/day produced a 100% inhibition, and intervening doses resulted in incremental inhibition of the decidual cell response (Cummings and Gray, 1987). Animals were weighed and killed by decapitation on Day 9 of pregnancy, at which time trunk blood was collected. Serum was separated in serum separation tubes by centrifugation, frozen, and later used for assay of serum progesterone by radioimmunoassay.[4] Uteri were removed, trimmed, and split at the cervical junction. Individual cornua were weighed to the nearest 0.10 mg. Implantation sites were counted by gross inspection and observed for size and presence of blood. Each pair of ovaries was trimmed, weighed, and fixed in 10% buffered formalin. Subsequently, corpora lutea were counted under binocular low magnification.

In a separate protocol, pregnant rats were treated with one of five regimens (Figs. 1A-2 and 1A-3): oil (vehicle) Days 1–8; 200 mg/kg/day MXC Days 1–3 and oil Days 4–8; oil Days 1–3 and 200 mg/kg/day MXC Days 4–8; 500 mg/kg/day MXC Days 1–3 and oil Days 4–8; or oil Days 1–3 and 500 mg/kg/day MXC Days 4–8. These regimens were designed to provide exposure to the pesticide strictly during either the preimplantation period, Days 1–3, or the period of postimplantation development, Days 4–8. The two doses were chosen on the basis of minimum and maximum effective dose for inhibition of the decidual cell response (Cummings and Gray, 1987). Animals were killed on Day 9, and tissues were analyzed as described above.

Pseudopregnancy protocol. Pseudopregnancy was induced by stimulation of the uterine cervix with a small brass rod on proestrus and estrus (Fig. 1B) (De Feo, 1963). Day 0 of pseudopregnancy was defined by vaginal cornification which was followed by leukocytic infiltration on Day 1. The decidual cell response (DCR) was surgically induced in both uterine cornua of each rat by the knife-scratch traumatization technique (TR) on Day 4 of pseudopregnancy between 10 AM and 12 noon (De Feo, 1963). This technique mimics the effect of an implanting blastocyst, induces the development of uterine decidual tissue, and normally results in a nearly 10-fold increase in uterine weight after 5 days of growth. Animals were treated during Days 1–3 with oil, 200 mg/kg/day MXC, or 500 mg/kg/day MXC with no treatment Days 4–8, or were treated Days 4–8 with oil, 200 mg/kg/day MXC, or 500 mg/kg/day MXC with no treatment Days 1–3 (Figs. 1B-1 and 1B-2). Rats were killed on Day 9 of pseudopregnancy exactly as described for pregnant animals. Tissue collection and analysis were also identical, with the obvious exception of the counting of implantation sites.

Ovariectomized, hormone-treated protocol. Groups of animals were bilaterally ovariectomized under ether anesthesia 2 to 3 weeks prior to use. Following 3 days of estrogen priming by sc injection of 5 μg/day estrone in 0.1 ml sesame oil and 1 day of no treatment (Day 0), rats were injected Days 1–8 with 1 μg estrone plus 2 mg progesterone per day (Fig. 1C). This regimen has been shown to support a maximal decidual response when uterine stimulation is performed on Day 4 (Cummings and Yochim, 1983). All animals underwent surgical decidual induction, as described above, on Day 4. One group of animals received 500 mg/kg/day MXC Days 1–8, while a second group received no additional treatment (Figs. 1C-1 and 1C-2). When the rats were killed on Day 9, uteri were removed, trimmed, and weighed.

Statistics. Data for all parameters were analyzed by the General Linear Models (GLM) procedure after logrithmic or arcsine transformation to correct for heterogeneity of variance (Helwig and Council, 1979; Sokal and Rohlf, 1981). When significant effects on the overall ANOVA were detected ($p < 0.05$), post hoc comparisons among treatments were made with t tests.

RESULTS

Early pregnancy: Dose response. When administered during early pregnancy (Days 1–8), 100 mg/kg/day of MXC did not significantly affect the mean number of implantation sites found on Day 9 as compared with vehicle-treated controls (Fig. 2A). By contrast, 200 mg/kg/day produced a significant decline in implantation site number ($p < 0.0001$), and 300, 400, and 500 mg/kg/day resulted in the complete abolition of implantations (Fig. 2A). MXC had no significant effect on uterine weight when administered at 100 mg/kg/day, but significant reductions occurred following 200, 300, 400, and 500 mg/kg/day ($p < 0.0005$; Fig. 2B). Similarly, the implantation index, calculated as (number of implantation sites/number of corpora lutea) × 100, was unaffected by 100 mg/kg/day MXC, but 200, 300, 400, and 500 mg/kg/day each produced a significant decline in this parameter ($p < 0.0002$; Table 1). Under the present protocol, MXC had no effect on

[4] Kit materials for radioimmunoassay of serum progesterone were obtained from Diagnostic Products Company, Los Angeles, CA.

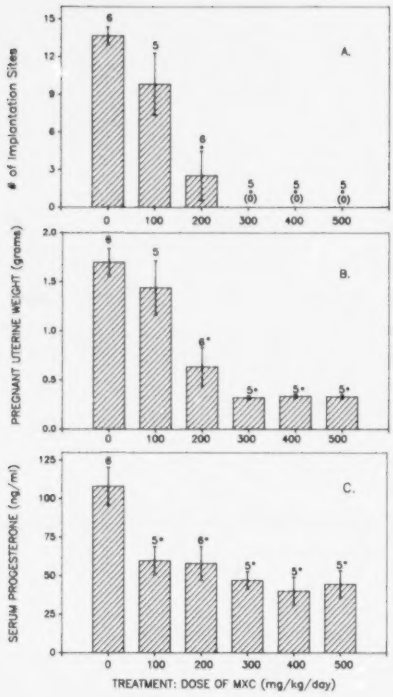

FIG. 2. Dose–response effect of MXC. Rats were dosed with vehicle or MXC at the indicated doses during early pregnancy, and animals were necropsied on Day 9. The values represent the means ± SEM, and the number of rats per group is shown above each bar. (A) MXC blocked implantation at 200 mg/kg/day and above. *Significantly different from vehicle control at $p < 0.0001$. (B) MXC produced a reduction in uterine weight at 200 mg/kg/day and above. *Significantly different from control at $p < 0.0005$. (C) MXC reduced serum progesterone at all doses used. *Significantly different from control at $p < 0.02$.

ovarian weight, mean number of corpora lutea, or body weight gain at any dose (Table 1). However, serum progesterone was sensitive to MXC treatment and exhibited a significant reduction at all doses when compared with vehicle control ($p < 0.02$; Fig. 2C).

Early pregnancy: Pre- versus postimplantation dosing. As shown in Fig. 3A, 200 or 500 mg/kg/day MXC had no effect on the mean number of implantation sites found on Day 9 if the compound was administered only during Days 4–8 of pregnancy. However, a dose-related effect was observed when MXC was given during Days 1–3. A dosage of 200 mg/kg/day produced a significant decline in implantation sites ($p < 0.015$), and 500 mg/kg/day reduced implantations to zero, an effect significantly different from that seen at the lower dose ($p < 0.0001$). Calculation of implantation index indicated a pattern identical to that seen for implantations alone (Table 2). Measurements of uterine weight showed a slightly different pattern (Fig. 3B). A significant, dose-related decline in uterine weight was observed when MXC was given during the preimplantation period, Days 1–3. However, 200 mg/kg/day MXC had no effect on uterine weight when administered during Days 4–8, while 500 mg/kg/day MXC administered during Days 4–8 produced a significant reduction in uterine weight as compared with control ($p < 0.001$). Uteri from all treatments showed some degree of resorption or inhibition of embryonic development as indicated by the presence of blood in or between implantations and the finding of implantation sites that were smaller than normal (Table 3). However, 100% of animals receiving 500 mg/kg/day MXC on Days 4–8 had uteri containing blood, and six out of seven animals had uteri containing at least some smaller than normal implantation sites. None of the treatments had a significant effect on either ovarian weight or number of corpora lutea (Table 2). However, a significant effect of MXC on serum progesterone concentration was observed (Fig. 3C), with the pattern of effects very different from that seen for implantations. While either 200 or 500 mg/kg/day during Days 1–3 had no effect relative to control, both dosages caused a decline in progesterone concentration when administered during Days 4–8 ($p < 0.05$). MXC had no effect on body weight gain at 200 mg/kg/day, but a significant reduction in this parameter was seen following 500 mg/kg/day MXC ($p < 0.01$; Table 2).

Pseudopregnancy: DCR. Analysis of the effect of MXC on uterine preimplantation

TABLE 1

EFFECTS OF MXC DURING EARLY PREGNANCY[a]

Treatment (mg/kg/day)	N	Ovarian weight (mg)	Number of corpora lutea	Body weight gain[b] (g)	Implantation index[c]
0 (Vehicle)	6	75.6 ± 14.9	15.7 ± 0.8	16.7 ± 0.8	87.4
100	5	56.5 ± 4.0	14.2 ± 1.0	8.9 ± 3.6	66.9
200	6	57.2 ± 9.1	14.3 ± 0.8	11.2 ± 2.0	19.8*
300	5	54.1 ± 2.9	14.4 ± 0.2	2.2 ± 6.9	0.0*
400	5	54.3 ± 3.7	14.8 ± 1.3	−3.0 ± 2.7	0.0*
500	5	53.2 ± 4.5	14.4 ± 0.5	8.4 ± 5.8	0.0*

[a] MXC was administered to groups of rats during Days 1–8 of pregnancy. All parameters were assessed following euthanasia on Day 9. N = number of rats per treatment. Data are expressed as means ± SEM, except for implantation index.

[b] Mean of the difference in body weight of each rat in each group between Day 1 and Day 9.

[c] (Number of implantation sites/number of corpora lutea) × 100. Each value is the mean of each group of rats.

* Significantly different from control group at $p < 0.0002$.

differentiation as compared with postimplantation proliferation and development was approached using the decidual cell response technique in a manner similar to that used previously for study of effects on early pseudopregnancy (Cummings and Gray, 1987). Doses of 200 mg/kg/day of MXC had no effect on uterine weight (decidual growth) when administered either during Days 1–3 or Days 4–8 of pseudopregnancy (Fig. 4). However, 500 mg/kg/day MXC inhibited the DCR and reduced uterine weights significantly when administered either prior to or after implantation (Fig. 4). In addition, the degree of DCR inhibition was more pronounced when the high dose of MXC was administered during the preimplantation period, Days 1–3 ($p < 0.004$), as compared with postimplantation dosing ($p < 0.0001$; Fig. 4). No significant effect on body weight was detected under the conditions of the protocol.

Ovariectomy and hormone treatment: DCR. In a final experiment, the DCR was induced in long-term ovariectomized rats treated with a regimen of estrogen and progesterone which supports uterine decidual development. As shown in Fig. 5, the administration of MXC at 500 mg/kg/day during 8 days of this artificial pseudopregnancy blocked the uterine growth characteristic of the DCR in the absence of any ovarian influence ($p < 0.05$).

DISCUSSION

In an earlier paper (Cummings and Gray, 1987), the estrogenically active MXC was shown to inhibit the DCR in a dose-dependent manner, providing evidence that the antifertility effect of the pesticide is mediated, at least in part, via the suppression of uterine decidualization, an action that is characteristic of estrogenic compounds (Yochim and De Feo, 1962, 1963). Data from the present study show a similar dose-related decline in the number of implantation sites, uterine weight, and implantation index, with no observable changes in ovarian weight, number of corpora lutea, or body weight gain. However, serum progesterone was significantly lower on Day 9 of pregnancy at all doses of MXC in contrast to the lack of effect of MXC on this parameter seen during early pseudopregnancy (Cummings and Gray, 1987). Serum progesterone levels in control rats were comparable to literature values (Pepe and Rothchild, 1974).

Luteal development and maintenance of progesterone secretion depend primarily on

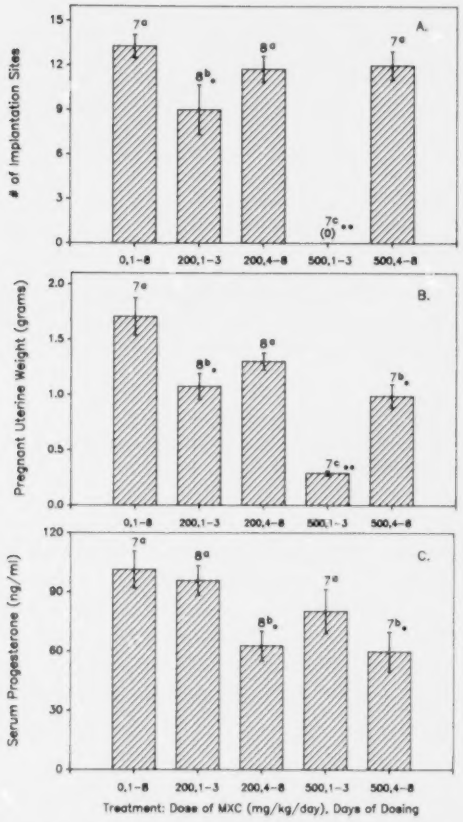

FIG. 3. Pre- versus postimplantation effects of MXC. Animals were administered MXC at the indicated doses either prior to (Days 1–3) or following (Days 4–8) implantation and killed on Day 9. Treatments are represented as dose of MXC (mg/kg/day), days of dosing during pregnancy. Each value represents the mean ± SEM; the number above each bar is the number of rats per group. Significant differences between groups are indicated by different letters (*a, b, c*). (A) Preimplantation MXC administration reduced implantations in a dose-related manner. *,**Significantly different from vehicle control at $*p < 0.015$ and $**p < 0.0001$. (B) Pregnant uterine weight was sensitive to both dosage and timing of administration of MXC. *,**Significantly different from vehicle control at $*p < 0.001$ and $**p < 0.0001$. (C) MXC reduced serum progesterone only when administered following implantation. *Significantly different from control at $p < 0.05$.

prolactin through Day 8 of pregnancy (Morishige and Rothchild, 1974). Exogenously applied estradiol has been shown to induce an earlier onset of luteal regression in rats bearing pituitary autografts (Ochai and Rothchild, 1985). The reported hastening of luteal regression was attributed to the estrogenic facilitation of the production of prostaglandins within the corpora lutea. These prostaglandins are luteolytic and inhibit progesterone secretion (Rothchild, 1981).

The measured reduction of serum progesterone by MXC may be unimportant, physiologically, for implantation. MXC administration at 100 mg/kg/day produced no decline in implantations but reduced serum progesterone. Doses of MXC producing reduced fertility (200–500 mg/kg/day) resulted in serum progesterone levels no different from those seen using the lower dose. Also, whereas implantation is complete by Day 5, serum progesterone was measured on Day 9; the hormone levels may have been adequate for implantation but affected sufficiently by Day 9 to produce resorption. Our data showing a blockade of uterine decidualization (DCR) by MXC in ovariectomized rats under controlled hormonal conditions suggest that the estrogenic pesticide was inhibitory in the presence of adequate progesterone and that no ovarian effect need be invoked for MXC action to interfere with the implantation process.

Another factor in MXC-induced infertility may be the acceleration of tubal transport rate of fertilized ova. In the rat, a single injection of estradiol cyclopentylproprionate (ECP) on Day 0 was shown to increase the tubal transport rate of fertilized ova, resulting in the interruption of pregnancy in over 80% of the animals given 10 μg ECP and in 50% of animals given 1 μg ECP (Greenwald, 1967). These data are consistent with a reduction in fertility by MXC under the current protocol, and further work is necessary to evaluate this phenomenon.

The most obvious mechanism contributing to MXC-induced infertility is the direct effect of the estrogenic material on the development of uterine sensitivity for implantation and the growth of decidual tissue. The

TABLE 2

EFFECT OF MXC DURING PRE- AND POSTIMPLANTATION DOSING[a]

Treatment[b]	N	Ovarian weight (mg)	Number of corpora lutea	Body weight gain[c] (mg)	Implantation index[d]
0; 1–8	7	58.6 ± 2.8	13.6 ± 0.7	23.3 ± 1.7	97.80
200; 1–3	8	57.0 ± 2.2	13.5 ± 0.5	20.4 ± 2.9	65.66**
200; 4–8	8	52.4 ± 2.6	12.9 ± 0.4	16.1 ± 2.2	90.98
500; 1–3	7	56.4 ± 2.0	13.3 ± 0.4	10.4 ± 2.2*	0.00**
500; 4–8	7	49.9 ± 1.5	12.7 ± 0.8	10.6 ± 3.3*	94.40

[a] MXC was administered to groups of rats during Days 1–3 or 4–8 of pregnancy, and parameters were assessed following euthanasia on Day 9. N = number of rats per treatment. Data are presented as means ± SEM, except for implantation index.

[b] Dose of MXC (mg/kg/day); days of dosing during pregnancy.

[c] Mean of the difference in body weight of each rat in each group between Day 1 and Day 9.

[d] (Number of implantation sites/number of corpora lutea) × 100. Each value is the mean of each group of rats.

* $p < 0.01$, ** $p < 0.001$ for comparisons between treatments and vehicle controls.

previously reported dose-related inhibition of the decidual cell response by both MXC and estrone (Cummings and Gray, 1987) suggests that such a direct mechanism participates in the fertility reduction seen when MXC was administered during early pregnancy. Although an ovarian-mediated mechanism cannot be ruled out in other protocols, the present finding of an inhibition of the DCR by MXC in hormone-maintained ovariectomized rats is strong evidence for the direct effect of the estrogenic compound on uterine function. MXC may have compromised the development of sensitivity to decidualiza-

TABLE 3

EVALUATION OF ABNORMAL IMPLANTATION SITES[a]

Treatment[b]	N	Rats showing blood at implantation sites[c]	Rats with smaller than normal sites[d]
0; 1–8	7	42.9 (3/7)	28.66 (2/7)
200; 1–3	8	25 (2/8)	50 (4/8)
200; 4–8	8	100 (8/8)	71.4 (5/7)
500; 1–3	7	0 (no sites)	0 (no sites)
500; 4–8	7	100 (7/7)	85.7 (6/7)

[a] Animals were treated as described in Table 2. N = number of rats per group.

[b] Dose of MXC (mg/kg/day), days of dosing during pregnancy.

[c] Percentage (proportion) of animals in which blood was observed in or between implantation sites.

[d] Percentage (proportion) of rats in which uteri contained at least some smaller than normal implantation sites.

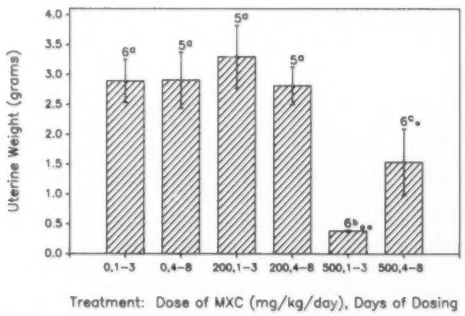

FIG. 4. Effect of MXC on the DCR: Pre- versus postimplantation. Rats were dosed with vehicle or MXC either prior to (Days 1–3) or following (Days 4–8) decidual induction during pseudopregnancy. Treatments are represented as the dose of MXC (mg/kg/day), days of dosing during pseudopregnancy. Each value represents the mean ± SEM; the number of rats per group is shown above each bar. Groups are labeled *a*, *b*, and *c* to indicate significant differences from each other. Inhibition of the DCR was sensitive to MXC dosage and timing of administration. ***Significantly different from vehicle controls at * $p < 0.0001$ and ** $p < 0.004$.

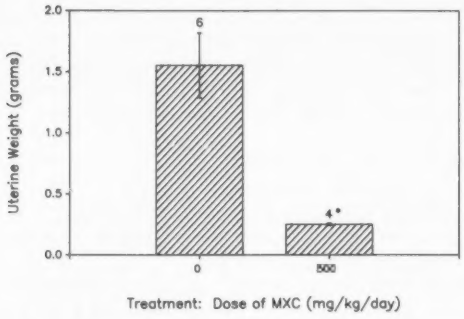

FIG. 5. Inhibition of the DCR by MXC in hormone-prepared, ovariectomized rats. Hormone-maintained, ovariectomized rats were administered MXC or no treatment for 8 days, and decidual induction was performed on Day 4. Values represent means ± SEM; the number above each bar is the number of rats per group. MXC blocked uterine decidualization in the absence of ovarian influence. *Significantly different from control at $p < 0.05$.

tion, blocked the induced decidual growth, or exerted both effects via its estrogenic activity.

A probe of a possible differential effect of MXC on preimplantation development versus postimplantation decidual growth revealed that 200 mg/kg/day, a dose that inhibited the DCR when given on Days 1–8 (Cummings and Gray, 1987), had no effect on the DCR when administered only pre- or postimplantation. These data and the finding of a DCR inhibition by MXC at 500 mg/kg/day which was greater during the preimplantation period demonstrated that preimplantation development is more sensitive to disruption by the pesticide than is the postimplantation decidual growth and that both pre- and postimplantation processes are sensitive to dose. This pattern is also true for estrone (Yochim and De Feo, 1962, 1963). These data also support a direct uterine mechanism for the antifertility effect of MXC.

When a similar dosing protocol was used during early pregnancy, the finding of a dose-related decline in implantation sites and uterine weight with no effect on serum progesterone, ovarian weight, or number of corpora lutea when MXC was administered only during the preimplantation period supports the concept of the exquisite sensitivity of the preimplantation uterus to disruption of development by exogenous estrogens. The fact that preimplantation administration of the lower dose reduced implantations but did not affect serum progesterone or the DCR may be attributed to an accompanying mechanism such as enhanced ovum transport rate. Although MXC administration during the postimplantation interval did not affect the number of implantation sites, ovarian weight, or number of corpora lutea at either dose, the observed reduction in uterine weight and serum progesterone at the higher dose accompanied by a 100% rate of resorption suggests a blockade of decidual growth and placental development due to excess "estrogen" and possibly insufficient progesterone. This is consistent with the finding of an inhibition of the DCR when 500 mg/kg/day MXC was administered on Days 4–8 of pseudopregnancy.

In summary, the data show that short-term dosing of rats with MXC during early pregnancy produces a significant and dose-related reduction in fertility. This reduction is seen partly as a direct disruption of the development of uterine receptivity for implantation and a compromise of the organ's capability for postimplantation decidual growth by the estrogenic pesticide. Other effects include a decline in serum progesterone due to an apparent acceleration of luteal regression and a possible, though undemonstrated, acceleration of tubal transport of the fertilized ova. Whereas dosing with MXC during the preimplantation period of pregnancy blocks implantation, postimplantation dosing produces fetal resorption. Possible mechanisms contributing to these effects include MXC's estrogenic influence on uterine preimplantation differentiation, ovum transport rate, luteal regression, and postimplantation decidual growth.

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